REMARKS

Entry of this Amendment, reconsideration and allowance of the above-captioned patent application are respectfully requested. This application relates to selective S1P1/Edg1 receptor agonists.

Status of Claims

Claims 1 to 61 are currently pending in the application. Claims 12, 15 to 18, 20 to 45 and 60 to 61 have been withdrawn from consideration as drawn to non-elected subject matter. Claims 1 to 11, 13, 14, 19 and 46 to 59 have been rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. Claims 1 to 11, 13, 14, 19 and 46 to 59 have been rejected under 35 U.S.C. 112, first paragraph, for non-enablement. Claims 1 to 11, 13, 14, 19 and 46 to 59 have been rejected under 35 U.S.C. 112, second paragraph, as being indefinite for lack of antecedent basis. Claim 1 has been provisionally rejected for non-statutory obviousness-type double patenting over claim 34 of co-pending application no. 10/500,895, claim 19 of co-pending application no. 10/554,665, and claim 14 of co-pending application no. 10/571,334. The abstract of the disclosure has been objected to. No claim has been allowed.

This Amendment amends Claim 6 and cancels Claims 1 to 5 and 12 to 61. Upon entry of this Amendment, Claims in the application will be Claims 6 to 11.

Canceled Claims

All rejections and objections pertaining to Claims 1 to 5 and 12 to 61 have been rendered moot by the cancellation of these claims. Applicants reserve the right to prosecute canceled subject matter in one or more future continuation or divisional applications.

Abstract of the Disclosure

Applicants have amended the specification to replace the Abstract of the Disclosure. In the new abstract, the words "said" have been replaced with the words --the--. Withdrawal of the objection to the Abstract of the Disclosure is respectfully requested.

Written Description

Claims 6 to 11 have been rejected for lack of written description for use of the term "immunoregulatory abnormality". Although Applicants disagree with the Examiner's

written description rejection, this term has been replaced in Claim 6 with the term --suppressing the immune system--. Support for this change is found, for example, at page 30, lines 28 to 31, and, as such, Applicants submit the written description requirement is fulfilled. One skilled in the art clearly understands the meaning of the term "suppressing the immune system" and the exact interpretation of this term can be fully ascertained. Withdrawal of the rejection for lack of written description under 35 U.S.C. § 112, first paragraph, is respectfully requested.

Enablement

As described above, Applicants have deleted the term "immunoregulatory abnormality" and replaced it with the term --suppressing the immune system-- in Claim 6. On page 4 of the Office action, the Examiner states that: "Claims 1-11, 13-14, 19 and 46-59 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for example 77, ... in the treatment of lymphopenia, does not reasonably provide enablement for all the disclosed compounds in the specification." Applicants respectfully disagree.

The specification in no way indicates the compounds disclosed therein are useful for the treatment of lymphopenia. The compounds of the invention are agonists of the S1P1 receptor which induce the sequestration of lympocytes. See page 2, lines 15 to 19. The assay described at page 161, beginning at line 19, assesses lymphopenia in mice to demonstrate the compounds are immunosupressants and not to treat lymphopenia. Thus, the specification fully enables the method of suppressing the immune system as currently claimed.

Furthermore, the specification provides enablement for more than Example 77. As stated at page 13, lines 9 to 14, the compounds described at pages 13 to 27 possess a selectivity for the $S1P_1$ receptor over the $S1P_3$ receptor of at least 20 fold and binding to the S1P1/Edg1 receptor of 100nM or less as evaluated by the ^{35}S-GTPγS Binding Assay. Applicants also refer the Examiner to J.J. Hale et al., *Biorganic & Medinical Chemistry Letters* 14 (2004) 2501-3505, a copy of which is submitted herewith. The work described therein helps to establish that the exploitation of structural factors that lead to the separation of affinities for $S1P_1$ and $S1P_3$ represent a practical approach for enhancing the cardiovascular tolerability of S1P receptor agonists. According to this paper, the severity of the acute toxicity for various test compounds in mice ranged from mild to severe independent of their selectivity for $S1P_1$ against either $S1P_4$ and $S1P_5$, while compounds that were at least 100-fold selective for $S1P_1$ against

$S1P_3$ and had absolute $S1P_3$ IC_{50} values greater that 100 nM were all better tolerated. See page 3504, first paragraph.

Applicants submit the claims as amended are fully enabled such that one having ordinary skill in the art could make and use the full scope of the claimed invention without undue experimentation. Withdrawal of the enablement rejection is respectfully requested.

Indefiniteness - Lack of Antecedent Basis

Applicants have amended Claim 6 to add a description of the assay entitled ^{35}S-GTPγS Binding Assay. The assay description refers to another assay entitled Ligand Binding to Edg/S1P Receptors Assay, and as such, a description of this assay has been added to Claim 6 as well. Support for this change is found at pages 151 to 154. Applicants submit this amendment overcomes the indefiniteness rejection of Claims 6 to 11 under 35 U.S.C. 112, second paragraph, for lack of antecedent basis. Withdrawal of this rejection is therefore respectfully requested.

Conclusion

Applicants submit that the application is in condition for allowance and passage thereto is earnestly requested. Any additional fees required in connection with this Amendment may be taken from Merck Deposit Account No. 13-2755. The Examiner is invited to contact the undersigned attorney at the telephone number provided below if such would advance the prosecution of the case.

Respectfully submitted,

By _____

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ABSTRACT OF THE DISCLOSURE

The present invention encompasses a method of treating an immunoregulatory abnormality in a mammalian patient in need of such treatment comprising administering to the patient a compound which is an agonist of the S1P$_1$/Edg1 receptor in an amount effective for treating the immunoregulatory abnormality, wherein the compound possesses a selectivity for the S1P1/Edg1 receptor over the S1PR3/Edg3 receptor, the compound administered in an amount effective for treating the immunoregulatory abnormality. Pharmaceutical compositions are included. The invention also encompasses a method of identifying candidate compounds that are agonists of the S1P$_1$/Edg1 receptor and which possesses a selectivity for the S1P1/Edg1 receptor over the S1PR3/Edg3 receptor. The invention further encompasses a method of treating a respiratory disease or condition in a mammalian patient in need of such treatment comprising administering to the patient a compound which is an agonist of the S1P$_1$/Edg1 receptor in an amount effective for treating the respiratory disease or condition, wherein the compound possesses a selectivity for the S1P1/Edg1 receptor over the S1PR3/Edg3 receptor.

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Selecting against $S1P_3$ enhances the acute cardiovascular tolerability of 3-(N-benzyl)aminopropylphosphonic acid S1P receptor agonists

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Abstract—Structurally modified 3-(N-benzylamino)propylphosphonic acid S1P receptor agonists that maintain affinity for $S1P_1$, and have decreased affinity for $S1P_3$ are efficacious, but exhibit decreased acute cardiovascular toxicity in rodents than do non-selective agonists.
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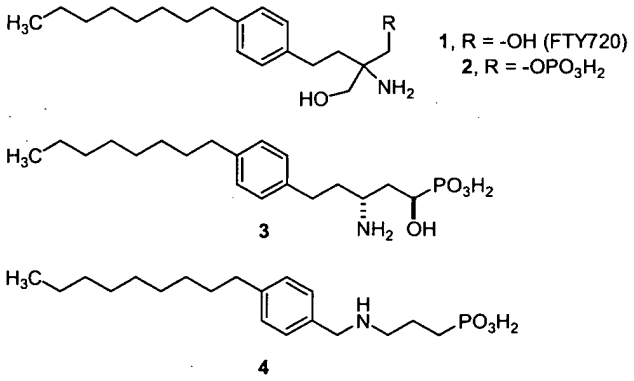
The immunosuppressive efficacy of FTY720 (**1**) has been proposed to result from its conversion in vivo to the corresponding phosphate ester (**2**), which is a potent agonist of four of the five known sphingosine-1-phosphate (S1P) receptors.[1,2] The systemic administration of **1** induces a dose–responsive lowering of circulating lymphocytes and the efficacy of **1** has been attributed to arise from this pharmacodynamic phenomenon.[3] The similarities observed for thymocyte emigration and lymphocyte circulation in mice with $S1P_1$ deleted from their hematopoietic cells and normal mice treated with **1** strongly indicates that an agonist-driven functional antagonism of $S1P_1$ is a required component in the mechanism of action of **1**;[4,5] several other recent reports have also connected the $S1P_1$ subtype to the efficacy of S1P receptor agonists.[6,7] The effectiveness of **1** in the clinic has been demonstrated in allogenic renal transplant patients[8] and this compound has progressed into Phase III trials.[9]



While **1** was reported to be well tolerated in Phase I clinical trials, a transient, asymptomatic bradycardia was an adverse event reported in 42% of the subjects.[10] During the work to identify the nonselective S1P receptor agonists **3** and **4**,[11,12] it was observed that these compounds and related analogs (as well as **2**) could cause an acute toxicity in mice that appeared to be

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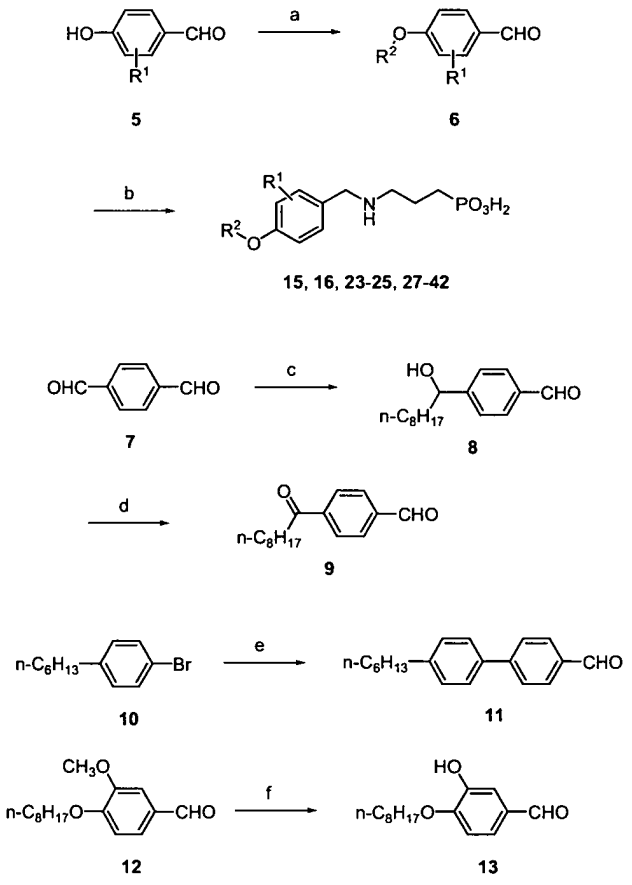
cardiovascular in nature. Since this toxicity was potentially mechanism based and seen with nonselective compounds, an obvious first step in attempting to circumvent it would be to determine whether the toxicity could be separated from the desired alterations in lymphocyte trafficking based on the agonism of different S1P receptors. We wish to report herein that specific structural alterations to 3-(N-alkyl)aminopropylphosphonic acid S1P receptor agonists[12] afford new analogs with high affinity for $S1P_1$, which correlates with their ability to lower circulating lymphocytes in rodents, but attenuated affinity for $S1P_3$, which correlates with an enhanced acute tolerability in the same. A subset of these new compounds has been employed as a tool in a definitive demonstration that the agonism of $S1P_3$ receptors can result in undesirable cardiovascular effects.[5]

The majority of the new S1P receptor agonist analogs are 3-(N-benzyl)aminopropylphosphonic acids and were synthesized using the previously described reductive amination procedure (Scheme 1).[12] Many of the benzaldehyde intermediates required for these reactions were obtained by simply O-alkylating the appropriate commercially available 4-(hydroxy)benzaldehyde. The benzaldehydes needed for alcohol **18** and ketone **17** were acquired by first alkylating terephthaldicarboxaldehyde (**7**) with *n*-octylmagnesium bromide followed by oxidation with Dess–Martin reagent.[13] The coupling of 4-(octyl)phenylbromide (**10**) and 4-(formyl)phenylboronic acid (required for analog **20**) was carried out using the conditions described by Buchwald.[14] Demethylation of **12** gave **13**, which was used to prepare **26**. The aldehydes needed to prepare tetrazole **21** and 1,2,4-oxadiazole **22** were obtained by modifying the respective literature procedures.[15,16]

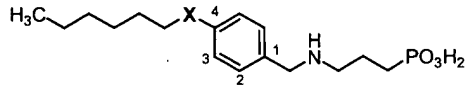


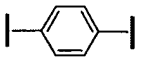
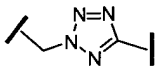
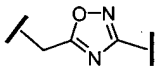
Scheme 1. Reagents and conditions: (a) R^2–X, K_2CO_3, CH_3CN, reflux (55–90%); (b) $H_2N(CH_2)_3PO_3H_2$, 1 equiv $Bu_4N^+OH^-$, $Na(CN)BH_3$, MeOH, 50 °C (20–40%); (c) *n*-$C_8H_{17}MgBr$, THF, 0 °C (5%); (d) Dess–Martin reagent CH_2Cl_2 (88%); (e) 4-(formyl)phenylboronic acid, KF, cat. $Pd(OAc)_2$, cat. 2-(dicyclohexylphosphino)-2′-methylbiphenyl, 1,4-dioxane, 80 °C (68%); (f) $BBr_3{\cdot}(CH_3)_2S$, CH_2Cl_2 (65%).

Ligand competition studies between [^{33}P]-S1P and all the new compounds were carried out for each of the five human S1P receptors stably expressed in Chinese Hamster Ovary (CHO) cell membranes.[1] S1P receptor agonism by the test compounds was also determined by measurement of ligand-induced [^{35}S]-5′-O-3-thiotriphosphate (GTPγS) binding; all of the compounds tested were found to be agonists of S1P receptors (data not shown).[17] No significant differences were observed for compounds tested in assays conducted with mouse or rat S1P receptors. The 3-(N-benzyl)aminopropylphosphonic acids in Table 1 were prepared to examine the effect of the group linking the phenyl ring to the pendant alkyl chain in analogs like **4** and **14**. It was quickly discovered that 4-alkoxy analogs of a proper chain length could have S1P receptor profiles similar to 4-alkyl analogs (compare **4** and **16**). Changing the linker to a ketone **17** decreased affinity for $S1P_1$ by 3-fold and for $S1P_3$ by almost 100-fold as compared to **4** indicating that this region of molecular space could be potentially exploited to enhance $S1P_1/S1P_3$ selectivity. Neither alcohol **18** nor ester **19** improved on the profile of **17**. Biphenyl analog **20** represents a different variation of the side chain linker; a subnanomolar $S1P_1IC_{50}$ value was maintained and its 200-fold selectivity for $S1P_1$ over $S1P_3$ is on par with that seen for ketone **17**. Heterocyclic analogs **21** and **22** were prepared as potential hybrids of **17** and **20**; while both compounds have increased affinity for $S1P_3$, they both maintain 100-fold selectivity for $S1P_1$ over $S1P_3$. Attempts to transpose some of the side chains of these analogs to the 3-position of the phenyl ring or to extend the scope of linker structures to include amides or sulfonamides gave analogs with greatly decreased affinity for all S1P receptors (data not shown).

The ready availability of intermediates for the preparation of analogs of **15** allowed for their in depth investigation (Table 2). For such analogs, the addition of small substituents (–Cl, $–OCH_3$) to the 2-position of the phenyl ring did not result in appreciable changes in overall S1P receptor affinity or selectivity (data not shown). For substituents added to the 3-position of the phenyl ring (**23–29**), electron-withdrawing groups appeared to be somewhat preferred for overall S1P receptor affinity while increasing the size of the group was found to influence $S1P_1/S1P_3$ selectivity as desired (compare methoxy analog **24** to **15** or the trend **27** to **28** to **29**). Doubly flanking the 4-octyloxy group with the same substituent could serve to enhance $S1P_1/S1P_3$ selectivity (compare **30** to **23**), but the compounds with the greatest

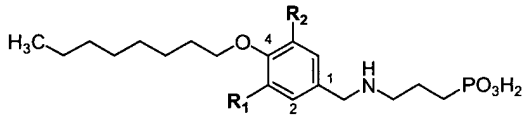
Table 1. Inhibition (IC_{50}, nM) of [^{33}P]-S1P binding to S1P receptors[a]



Compd	X	$S1P_1$	$S1P_2$	$S1P_3$	$S1P_4$	$S1P_5$
4	$-CH_2CH_2CH_2-$	0.2	750	2.7	40	0.7
14	$-CH_2CH_2-$	0.6	>10,000	21	26	5.5
15	$-CH_2CH_2O-$	1.5	4600	8.5	530	6.3
16	$-CH_2CH_2CH_2O-$	0.3	840	1.1	74	1.7
17	$-CH_2CH_2(C{=}O)-$	0.9	8600	260	250	4.0
18	$-CH_2CH_2CH(OH)-$	32	5400	110	2100	110
19	$-CH_2O(C{=}O)-$	2.3	>10,000	280	700	680
20		0.9	3400	180	120	2.6
21		0.5	2300	55	270	1.4
22		0.5	1500	46	120	1.8

[a] Displacement of [^{33}P]-labeled sphingosine-1-phosphate (S1P) by test compounds from human S1P receptors expressed on CHO cell membranes. Data are reported as mean for $n = 3$ determinations. SD were generally ±20% of the average. See Ref. 1 for assay protocol.

Table 2. Inhibition (IC_{50}, nM) of [^{33}P]-S1P binding to S1P receptors[a]



Compd	R_1	R_2	$S1P_1$	$S1P_2$	$S1P_3$	$S1P_4$	$S1P_5$
15	–H	–H	1.5	4600	8.5	530	6.3
23	$-CH_3$	–H	0.5	>10,000	6.0	32	1.8
24	$-OCH_3$	–H	1.9	>10,000	99	450	24
25	–OEt	–H	2.5	>10,000	74	420	12
26	–OH	–H	14	>10,000	110	1200	41
27	–F	–H	0.7	4700	1.2	59	2.0
28	–Cl	–H	0.3	>10,000	4.9	55	1.7
29	–Br	–H	0.4	>10,000	26	36	2.9
30	$-CH_3$	$-CH_3$	0.4	>10,000	44	15	0.8
31	–Cl	–Cl	0.6	>10,000	13	6.6	1.0
32	–Br	–Br	1.3	>10,000	94	23	3.0
33	$-CH_3$	$-OCH_3$	2.3	>10,000	1000	89	19
34	–Cl	$-OCH_3$	2.4	>10,000	610	57	8.0
35	–Br	$-OCH_3$	4.1	>10,000	2100	80	10

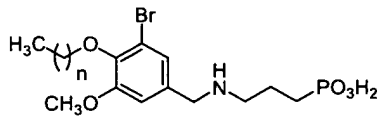
[a] Displacement of [^{33}P]-labeled sphingosine-1-phosphate (S1P) by test compounds from human S1P receptors expressed on CHO cell membranes. Data are reported as mean for $n = 3$ determinations. SD were generally ±20% of the average. See Ref. 1 for assay protocol.

window were obtained on combining 3-substituents previously found in analogs with enhanced $S1P_1$ affinity as compared to **15** with the selectivity-enhancing methoxy group of **24**. Compounds **33–35** all exhibit 250–500-fold selectivity for $S1P_1$ over $S1P_3$ with the $S1P_3IC_{50}$ values for **33** and **35** being 1–2 μM.

A factor that influenced S1P receptor affinity in analogs of **4** was the overall length of the lipophilic region.[12] The compounds in Table 3 are straight chain ether analogs of **35** in which the alkyl group ranges from 6 to 11 carbon atoms. Overall affinity for all S1P receptors (except for $S1P_2$) does correlate with increased alkyl chain length for these compounds, but this occurs at the cost of losing 3-fold in selectivity for $S1P_1$ over $S1P_3$ on going from hexyl ether **36** to undecyl ether **40**. Regardless, analogs such as **35**, **38**, and **39** maintain good affinity for $S1P_1$ while being the most selective against $S1P_3$ prepared as part of this work.

New compounds were screened for their ability to lower circulating lymphocyte levels in mice as a surrogate marker for immunosuppressive efficacy.[11] Simple ether analogs **15** and **16** and substituted octyl ethers **23**, **24**, and **27–31** were all found to be lethal 1–2 min after the administration of 4 mpk iv doses as was previously observed with **4**.[12] Lower doses given via the peritoneal cavity were better tolerated and several of these

Table 3. Inhibition (IC_{50}, nM) of [^{33}P]-S1P binding to S1P receptors[a]



Compd	n	$S1P_1$	$S1P_2$	$S1P_3$	$S1P_4$	$S1P_5$
36	5	7.3	6500	8200	260	6.2
37	6	13	>10,000	>10,000	33	6.1
35	7	4.1	>10,000	2100	80	10
38	8	1.4	>10,000	830	33	3.9
39	9	0.4	>10,000	200	18	1.5
40	10	1.2	>10,000	430	42	1.9

[a] Displacement of [^{33}P]-labeled sphingosine-1-phosphate (S1P) by test compounds from human S1P receptors expressed on CHO cell membranes. Data are reported as mean for $n = 3$ determinations. SD were generally ±20% of the average. See Ref. 1 for assay protocol.

compounds (**15**, **23**, **31**) were found to induce a maximal lymphocyte lowering response 3 h after an 0.1–0.25 ip dose. The severe toxicity was found to be attenuated when ketone **17**, biphenyl analog **20**, and dibromide **32** were screened while no visible signs of distress were seen when ethers **33–40** were given similarly. Dose–titration data for circulating lymphocyte reduction for selected compounds is shown in Table 4; the ED_{50} values are consistent with the trend observed for the $S1P_1$ IC_{50}s. These new compounds did not exhibit appreciable oral bioavailability in rodents, however pharmacokinetic data for **20**, **32**, and **35** in the rat indicate that many of these compounds would be expected to produce comparable plasma coverage after iv administration.

The severity of the acute toxicity for various test compounds in mice ranged from mild to severe independent of their selectivity for $S1P_1$ against either $S1P_4$ and $S1P_5$ (e.g., compare severely toxic **15** to mildly toxic **20** or mildly toxic **23** to well-tolerated **35**), while compounds that were at least 100-fold selective for $S1P_1$ against $S1P_3$ and had absolute $S1P_3$ IC_{50} values greater than 100 nM were all better tolerated. Two sets of experiments were conducted to support the supposition that these subjective observations were based on selection against $S1P_3$.[5] First, iv bolus administration of compounds with different degrees of $S1P_1/S1P_3$ selectivity to anesthetized Sprague–Dawley rats resulted in a bradycardia that could be readily monitored. The extent of the bradycardia, measured as the peak decrease in heart rate as a percentage change from average baseline rate, was found to be dose–responsive for compounds **2**, **15**, **35**, and **36** with decreased $S1P_3$ affinity correlating with the higher doses required to elicit similar absolute heart rate lowering responses for the various compounds. Second, administration of a 1.0 mpk iv bolus of the nonselective agonist **15** to male $S1P_3$ null mice was found to elicit a maximal lymphocyte response at a 3 h time point and was well tolerated. A similar experiment in wild-type mice was found to result in death within 90 s. Taken as a whole, these data indicate that $S1P_3$ receptor agonism is not required for efficacy, but does play a significant role in the observed acute toxicity of nonselective S1P receptor agonists.[19]

Table 4. Mouse peripheral lymphocyte lowering[a] (PLL) and rat pharmacokinetic[b] data for selected S1P receptor agonists

Compd	Murine PLL ED_{50} (mpk iv)	Rat PK (1.0 mpk iv)
17	0.4	ND[c]
20	0.3	$Cl_p = 4.6$ mL/min/kg $Vd_{ss} = 1.8$ L/kg, $t_{1/2} = 1.9$ h
32	0.6	$Cl_p = 8.3$ mL/min/kg $Vd_{ss} = 1.0$ L/kg, $t_{1/2} = 2.6$ h
35	2.1	$Cl_p = 9.2$ mL/min/kg $Vd_{ss} = 1.4$ L/kg, $t_{1/2} = 2.1$ h

[a] Individual data points for dose–titrations were the average percentage decrease of peripheral blood lymphocyte counts in $n = 3$ animals versus control ($n = 3$) 3 h after iv administration of the test compound. SD were generally ±20% of the average. See Ref. 11 for assay protocol.

[b] See Ref. 18.

[c] Not determined.

In conclusion, several reports have appeared that strongly implicate $S1P_1$ receptors in the immunosuppressive efficacy of **1**,[4–7] while a more complete understanding of the undesirability of $S1P_3$ receptor agonism is just emerging.[6,7,20] It has been shown that appropriately substituted 3-(N-benzyl)aminopropylphosphonic acid S1P receptor agonists can maintain high affinity for $S1P_1$, but have decreased affinity for $S1P_3$ with the steric constraints imposed by the added substituents appearing to be the basis for the observed selectivity. Such changes resulted in efficacious analogs that were less acutely toxic in rodents. This work helps to establish that the exploitation of structural factors that lead to the separation of affinities for $S1P_1$ and $S1P_3$ represents a practical approach for enhancing the cardiovascular tolerability of S1P receptor agonists. The identification and characterization of S1P receptor agonists with enhanced selectivity and pharmacokinetic profiles will be the subject of future reports.

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17. All test compounds were found to be agonists of human $S1P_1$ $S1P_2$, $S1P_3$, $S1P_4$, and $S1P_5$ receptors as evidenced by their ability to induce levels of GTPγS binding comparable to S1P. The magnitudes of the calculated EC_{50} values from these assays were generally +/−5-fold of the IC_{50} values.
18. Plasma compound concentration measurements used to calculate pharmacokinetic parameters were obtained after iv administration of test compounds via a cannula that had been previously implanted in the femoral vein of male Sprague–Dawley rats ($n = 2$). Compounds **2** and **6** were formulated at 1.0 mg/mL in 2% hydroxymethyl-β-cyclodextrin/10 nM Na_2CO_3.
19. Due to the connections between $S1P_1$ and immunosuppressive efficacy, a successful receptor selectivity-based separation of efficacy and acute toxicity requires that the latter be driven by a receptor other than $S1P_1$; the data reported here support the involvement of $S1P_3$. Regarding the other S1P receptor subtypes, $S1P_2$ can be definitely ruled out based on the negligible affinity that many acutely toxic S1P receptor agonists have for this receptor, while the more limited expression patterns of $S1P_4$ (to lymphoid and hematopoietic tissues) and $S1P_5$ (to the central nervous system)[21] would suggest it unlikely that those receptors play roles in the observed toxicity.
20. S1P-induced activation of muscarinic receptor-activated inward rectifier K^+ current in cultured mouse, guinea pig, and human atrial myocytes appears to be mediated by $S1P_3$. See: Himmel, H. M.; Heringdorf, D. M. Z.; Graf, E.; Dobrev, D.; Kortner, A.; Schüler, S.; Jakobs, K. H.; Ravens, U. *Mol. Pharm.* **2000**, *58*, 449–454.
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